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Application of Ph Indicator Label Based on Natural Pigments From Tomato for Determination of Milk Spoilage

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Developing an innovative indicator in packaging of food products can be essential to tell end- consumers about the quality, spoilage, and freshness of food products. In this research, new labels for monitoring the spoilage of milk were developed by extracting the natural pigments from tomatoes. One label prepared by using cellulose film and other label with chitosan film. The main purpose was to evaluate the abilities of these pH sensitive labels for monitoring spoilage of milk over time by establishing a correlation between pH variations and the label's apparent color changes. Labels characteristics were investigated by estimating label release test, film thickness, swelling index %, tensile strength and elongation at break percentage %. In comparison to cellulose-based labels (47.64 ± 1.40 MPa and 44.70 ± 1.12%, respectively), chitosan-based labels demonstrated greater tensile strength (56.20 ± 0.81 MPa) and elongation at break (49.50 ± 0.943%). In contrast to chitosan (0.22 ± 0.01), cellulose labels released less dye into milk (0.20 ± 0.01), suggesting that natural pigments were better retained, and that milk quality was less affected. These results show that cellulose labels have more control over pigment release, but chitosan labels are more resistant. Both films maintained their functionality and structural integrity, which made them appropriate for short-term use in dairy products. The total bacterial count, pH, lactic acid concentration in milk were measured using standard plate count, pH meter and titration of acidity on days 1, 3, 5, 7 at refrigerator temperature. Over the period of seven days, the chitosan label changed from orange-red to brown, and the cellulose label changed from pink to brown, both of which were apparent indications of milk spoilage. By comparing color changes with pH meter data, the accuracy of the pH detection was confirmed. The results indicated a positive correlation (r = 0.95) between color of label and variations in pH. All these findings demonstrate how these affordable, environmentally friendly labels may improve consumer safety by giving real-time milk freshness information.

Keywords: Intelligent packaging, synthetic dyes, spoilage indicator, carcinogenicity, antioxidant.

INTRODUCTION

Through the previous years, the concern about the safety and quality of food has increased. Therefore, the use of different types of sensors has been increasing for the packaging of foods to determine the food spoilage. With the advancement of different smart packaging, the food products spoilage can be sensed easily. The use of intelligent packaging has transformed the food packaging industry completely (Eshaghi *et al*., 2020).

Different methods of intelligent packaging are usually used for quick detection of spoilage in packed food products. (Priyadarshi *et al*., 2021). Indicators are tools or devices that indicate the presence and absence of various analytes via different ways, such as changes in color (Pirsa *et al*., 2022). A method which involves the placement of pH indicators in packaging indicates the food quality at the time of purchasing and after that, avoiding the consumption of spoiled foods (Balbinot-Alfaro *et al*., 2019).

In the past, mostly indicator films were made from synthetic dyes, which are carcinogenic by nature and have a negative impact on the health of consumers. Now consumers are fully aware of the carcinogenicity of the synthetic dyes, and as a result of it, more attention is provided on the application of natural colorants as compared to artificial colors (Almasi *et al*., 2022).

These natural colorants consist of anthocyanins, chlorophyll, curcumin, carotenoids, and betalains. From all these pigments, the lycopene contains a large number of plant-based dyes present in large quantities in different plant parts (Li *et al*., 2021). *Lycopersicon esculentum,* which is identified

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as tomato, contains lycopene in large amounts and is a key antioxidant (Sharma *et al*., 2021).

Lycopene is a pigment that is lipophilic in nature and exists in the form of red-shaded carotenoid dye, containing eight units of isoprene. The lycopene form of isomeric is associated directly with its color. The all-other lycopene isomers that are trans are red, while tetra-cis-lycopene isomers retain a shade of orange (Ghadage *et al*., 2019).

Milk is a highly perishable commodity, and its shelf-life stability is dependent on its conditions of storage and making it a suitable food for the formation of a freshness and spoilage indicator (Weston *et al*., 2020). Dairy products are included in those food groups which act as a perfect medium for microbial growth and thus result in spoilage and cause the sickness of end-users (Mirza Alizadeh *et al*., 2022).

Due to the spoilage of milk, there is a production of several microbial growth metabolites, which could result in unwanted changes in milk taste, flavor, and odor. The initial microbial amount, its distribution, and type have a great effect on the milk shelf-life period (Ziyaina *et al*., 2020). Usually, the quality of milk is evaluated in industrial laboratories by means of common methods that are standard plate count (SPC). All these methods are time-consuming and must be performed by trained staff in industrial microbiological laboratories (Eshaghi *et al*., 2020). Thus, several studies on various food quality indicators were carried out. The color-changing pH indicators may be used in milk and dairy products (Eshaghi *et al*., 2020).

Some scientists also developed films or labels derived from the mixture of quaternary ammonium chitosan/fish gelatin combined with betalain-rich extracts from amaranth to monitor the shrimp freshness kept at 20°C for 48 hours (Hu *et al*., 2020). According to research on the formation of anthocyanin-incorporated polymeric films, which have the pH indicator ability for determining the freshness of pork, the deterioration of pork was determined by the rise in the pH of headspace and total volatile basic nitrogen throughout storage (Zhang *et al*., 2019). At a limit of detection (LOD) of 0.81 μM, different research produced silver nanoparticles and spread them in a gellan gum carrier for the hydrogen sulfide detection in chicken breast (Zhai *et al*., 2019).

The growth of psychrotrophic bacteria, coliforms, lactic acid bacteria, and spore-forming organisms, which break down milk components and cause unwanted sensory changes, is the primary cause of microbial milk spoilage. Temperature, pH, initial microbial load, and availability of nutrient are some of the factors that greatly affect the rate of spoilage of milk; with improper handling and cross-contamination are also major contributors (Doyle *et al*., 2015).

Research indicating that even small changes in storage temperature can greatly accelerate the growth of microorganisms (Fusco *et al*., 2020). Therefore, it is essential to have a basic monitoring system in place that alerts customers to the safety of milk. A novel milk freshness label with tomato-colored components and several polystyrene layers was introduced in the current research.

MATERIALS AND METHODS

***Procurement of raw material*:** Chemicals and materials necessary for the production of the label were procured from the local market in Faisalabad.

***Preparation of label*:** A solution was prepared by adding 10 ml of lycopene extracted from tomato to 30 ml of distilled water. Placed it on a hot plate until it was dissolved completely. The alum solution was prepared by dissolving 10g of alum powder in 25 ml of distilled water at a temperature of 100°C. A polystyrene solution was prepared by introducing 1.8g of polystyrene powder into 15 ml of xylene. After that, these strips were immersed in a lycopene solution and heated for 30min. Afterward heating, cellulose or chitosan paper strips were introduced into an alum solution and subjected to a 30 min heating period. Labels were cut into smaller segments and immersed in a polystyrene solution and then dried it (Eshaghi *et al*., 2020).

Analysis of milk freshness

***Milk pH analysis*:** The milk pH was measured by using pH meter after buffer solutions for accuracy according to the AOAC (2019). pH meter was powered on and then its electrode was rinsed with distilled water. After distillation, the washed electrode was dipped in buffer solution of 4 pH and rinsed again with distilled water. Subsequently the pH meter electrode was dipped in buffer solution of 7 pH. As the pH meter gave an OK signal after completion of its calibration, it was again rinsed with distilled water. After that the electrode was dried with tissue paper. As calibration completed then about 50ml of 2 milk samples which are taken from those milk that contain pH films was taken in flasks and electrode was dipped in it for 3 times and each time washed and dried electrode respectively. Then readings were noted and took the average.

Microbial and acidity analysis of milk

Microbial analysis of milk

***Total plate count*:** Total bacterial count of milk was observed by plate count agar standard methods in AOAC (2019). In order to count total plates, the following actions were taken. In the autoclave, glassware was sterilized at 121°C for the duration of 90 minutes. The media was also sterilized in that autoclave at the same conditions. The test tube was filled with 9 ml peptone water then 1 ml sample was added in first test tube. Then dilution was done as 1 ml mixer of first test poured in the second test tube and this process was continued till 9 dilutions. Pouring medium was done on sterile petri dishes. 50 milliliter of sample was taken from each dilution, and decantation was performed in the middle. The samples were equally distributed in each petri dish after gently mixing with the spreader. In each petri dish, solidification took place. For one day, at 37°C petri dishes were placed in an incubator

along with the condensation prevention and inversion of these dishes were also done at the same time. The incubation procedure was finished after 24 hours and the colonies developed in petri dishes were counted using a colony counter. The total plate count was determined by the formula below:

Total plate count= Total plate count dilution factor x Average number of colonies

***Milk acidity analysis*:** The milk acidity was measured by the method of titration according to the AOAC (2019). The lactic acid concentration was determined by titrating with 0.1M of sodium hydroxide. Burette was filled with N/10 NaOH. Milk sample of 10 ml was transferred into a beaker and poured about 2-3 drops of indicator in milk. After that milk was titrated against 0.1 N NaOH solutions and noted initial volume. Titration was stopped when very light pink color appeared and noted the final reading. The acidity of the milk sample was determined by the formula below:

Acidity % = (volume of NaOH used×0.009/ volume of milk used) × 100

Labels analysis

***Label release test*:** According to Eshaghi *et al*. (2020), the detection of colour release on the seventh day was accomplished through the utilization of a UV spectrophotometer. To facilitate the separation of proteins and fat in milk, a milk sample measuring 30 µl was combined with 3 ml of ethanol. The centrifugation process was conducted on the mixture at a force of 4500 g for duration of 15 minutes. Subsequently, the resulting transparent liquid was shifted directly into a cuvette within the spectrophotometer apparatus, and the absorbance was quantified at a wavelength of 450 nm. The ultimate measurement of release of colour into the sample of milk with a complete label was determined by relating it to the values of absorption of the negative control (sample of milk lacking a label of freshness on the package, resulting in 0% release of dye into the milk) and the positive control (sample of milk containing a label of freshness but lacking polystyrene layers and white alum, resulting in 100% release of dye).

***Films thickness*:** Hand-held digital micrometer was used to measure the films thickness by following the standard method described by Halasz and Csoka (2018). The films' thickness was measured at various random positions. Three replications of each film were taken and then determined by its average.

***Swelling index %*:** The determination of the Swelling Index was conducted by using the method described by Maciel *et al*. (2015), with adjustments made to the time interval as per the standard procedure. Initially, the label samples were divided into pieces measuring 4.0 cm^2. Subsequently, the samples were placed in a desiccator having silica-gel for duration of 7 days. Subsequently, the samples were measured in terms of weight and subsequently immersed in beakers filled with 250 mL of distilled water for varying durations (0.5 min, 1 min, 3 min, 5 min, 7 min, 10 min, 15 min, and 20 min) at ambient temperature (25°C). Samples were extracted, subjected to a drying process, and subsequently measured in terms of weight at regular intervals.

The calculation of the Swelling Index (SI %) was performed using the following formula:

$$SI\% = \frac{(Wt - Wo)}{Wo} \times 100$$

Where W_t represents the weight of the swollen film at time t, and W_o represents the weight of the original film. The experiments were carried out in triplicate, and the resulting data were subsequently averaged.

***Tensile strength and elongation at break (%)*:** The tensile strength (TS) and elongation at break (EB) were assessed using a Testing Machine, employing a procedure outlined in the work conducted by Chen *et al*. (2020). The films were divided into strips of rectangular shape of measuring length of 40 mm and width of 20 mm. The grip separation and cross head speed were initially configured to be 20 mm and 0.6 mm/s, respectively. The measurements are indicative of the mean value obtained from three individual samples. The values for the TS and EB were determined using the following mathematical equations:

$$TS = \frac{Fmax}{S}$$

$$EB\% = \frac{\Delta l}{lo} \times 100$$

In this study, the variable TS represent the tensile strength, measured in mega Pascals (MPa). F_{max} denotes the maximum load applied to the sample of film, measured in newtons (N). Lastly, S represents the initial cross-sectional area of the film sample, measured in millimetres (mm).

The parameter denoted as EB represented the elongation at break, while Δl referred to the extension of the films measured in millimetres (mm). Additionally, Δl denoted the initial length of the films, also measured in millimetres (mm).

***Proximate analysis*:** Milk compositional analysis was done according to the standard methods in AOAC (2019).

***Crude fat content*:** The crude content of fat of milk was measured by the method of Gerber Centrifuge as stated in standard methods of AOAC (2019). The milk sample, with a temperature of approximately 20°C, was subjected to thorough mixing while ensuring minimal air incorporation. The sample was given a brief period to allow for the dissipation of any air bubbles, after which it was gently mixed once or more prior to pipetting. Subsequently, 10 ml quantity of sulphuric acid was introduced into the butyrometer, followed by the measured milk volume being transferred into the same apparatus using a pipette. Care must be taken to prevent the milk from charring. This can be achieved by ensuring a gentle flow of milk along the inner surface of the butyrometer, followed by placing it carefully on top of the acid. A volume of 1 millilitre of amyl alcohol was dispensed, and subsequently, the neck of the butyrometer was cleansed using a dry cloth. Butyrometer was stoppered firmly inserting

a clean and dry stopper. The butyrometer was agitated and inverted multiple times until complete absorption of the milk into the acid was achieved. The butyrometer was subjected to a temperature of 65°C for duration of 5 minutes within a water bath, followed by centrifugation at a speed of 1100 revolutions per minute for 5 minutes' duration. The butyrometer was placed back into the water bath for 5 minutes' duration, confirming that the level of water was sufficiently elevated to facilitate the heating of the fat column.

***Crude fiber content*:** Milk crude fiber content was done by the Gravimetric method as reported in standard methods of AOAC (2019). Fat free milk samples were used to assess the crude fiber content, which was then digested in 1.25 % Sulphuric acid (H_2SO_4). After that they were filtered and then washed three times. These milk samples were then digested with 1.25% sodium hydroxide (NaOH) for 30 minutes. Filtration and washing were also given thrice. The resulting filtrate then dried out at 130ºC temperature for about 2 hours and after that, they were weighed. Then they were incinerated at a high temperature (550ºC ± 15ºC).

$$\% \text{ Crude fiber} = \frac{\text{digested sample weight - ashed sample weight}}{\text{Sample weight}} \times 100$$

***Crude protein content*:** Kjeldhal's method was applied to measure the crude protein content of the milk as described in standard methods of AOAC (2019). A milk sample of 10 mL was collected in a digestion flask. A digestion mixture of total 5 grams consisting of 100g of K2SO4, 10g of CuSO4, and 5g of FeSO4 was added to the digestion flask. Subsequently, 35 millilitres of sulphuric acid were introduced into the digestion flask, which was then transferred to a fume hood for the purpose of digestion till the mixture attains a slightly green colour. The mixture was permitted to cool for a period, after which added a volume of 150ml of distilled water. The digestion flask underwent a rinsing process, consisting of two or three repetitions, to ensure the complete removal of the digested sample from within the flask. Following the process of digestion, distillation was conducted by combining 10ml of the digested sample with 10ml of NaOH (40%) in a conical flask. The distillate was obtained through the addition of a 10 mL boric acid (4%) solution while utilizing methyl red in form of indicator. The distillation process was extended for duration of 2-4 minutes after the transition of boric acid's colour from red to yellow, till stopped the bubbling and the maximum amount of ammonia was trapped. The residual substance present in the flask after the process of distillation was subjected to titration using a 0.1N H2SO4 solution until the appearance of a pink colour. The equation employed for the determination of the crude protein content in milk was as follows:

$$\% \text{ Nitrogen} = \frac{\text{Vol. of H2SO4 used (ml)} \times 250 \times 0.0014}{\text{Vol. of sample} \times \text{Sample used for distillation}} \times 100$$

Total Protein (%) = % Nitrogen × 6.38

Physicochemical analysis

***Specific gravity of milk*:** Milk specific gravity was analyzed by lactometer as described in standard methods of AOAC (2019). The milk sample was subjected to a 40°C temperature and maintained at this temperature for duration of 5 minutes. The purpose of this process was to transform all the fat into a liquid state due to the distinct density disparity between crystalline fat and liquid fat. Additionally, it was observed that fat exhibited a slow crystallization or melting rate. Following duration of 5 minutes, the milk underwent a cooling process resulting in a temperature of 20°C. Subsequently, the milk sample was subjected to a thorough yet gentle mixing procedure. Failure to shake the mixture vigorously may result in the incorporation of air bubbles, potentially impacting the outcome. The milk was introduced into the cylindrical container. The lactometer was held by its tip and carefully immersed into the milk until equilibrium was reached. The lactometer was permitted to achieve a state of equilibrium by allowing it to float without any external constraints. Subsequently, the lactometer was observed by reading the measurement at the peak point of the meniscus. The temperature of the milk was promptly measured. The desired temperature should be set at 20°C. To determine the value of L, correction factors were applied when the temperature of the milk fell within the range of 17°C to 24°C.

***Lactose content in milk*:** Milk lactose content was measured by Titrimetric method as stated in standard methods of AOAC (2019). 10ml of milk was taken in flask. 40ml of water was added in it. 8-9 drops of 5% acetic acid were added, precipitation occurred. It was filtered through filter paper. Filtrates were taken and make solution up to 100ml, and this filtrate was filled in burette. 5ml Fehling A and 5ml of Fehling B was take in conical flask. 20ml of water was added in it and 1-2 drops of methylene blue indicator were added. Titrated it with filtrate that was taken in the burette till brick red end point. The following formula was applied to measure the lactose content in milk:

$$\text{Lactose } \% = \frac{0.65 \times \text{D. F}}{\text{Vol. of filtrate used} \times \text{Vol. of sample}} \times 100$$

***Statistical analysis*:** Statistical analysis was performed in which proximate and physicochemical analysis analyzed via using software Minitab through two-way ANOVA general linear model on a 95% significance level ($\alpha = 0.05$) and labels analysis analyzed by one way ANOVA by the method defined by Montgomery (2017).

RESULTS

The current research aimed to develop pH indicator labels based on natural pigments from tomato for determination of milk spoilage. There were 2 treatments per storage studies and pH indicator labels were analyzed on 1, 3, 5 and 7 days.

Different tests of prepared labels, pH, acidity, total plate count and different milk tests were performed at different intervals to check the performance and effects of label on mi.

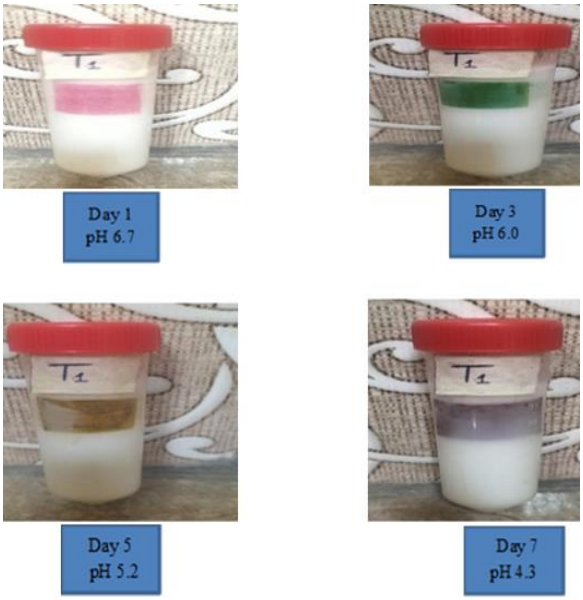

Figure 1. Cellulose containing label.

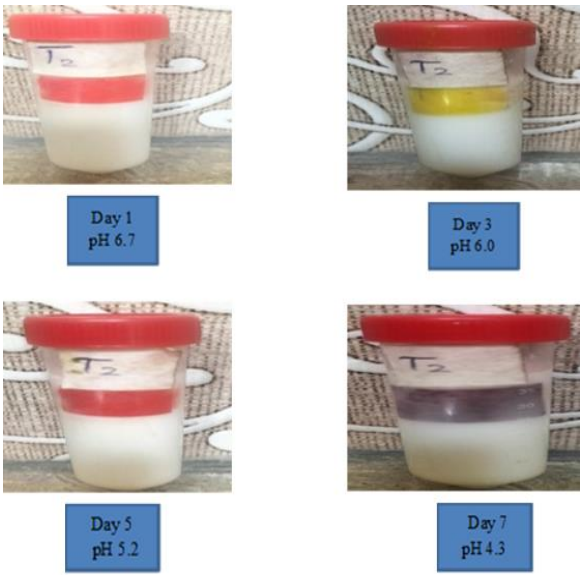

Figure 2. Chitosan containing label.

***Analysis of milk freshness*:** The fabricated indicator developed from cellulose and chitosan film changed its color due to change in pH. pH of fresh milk was 6.7 at the 1st day of storage, the color of the cellulose label was pink and when its pH decreased from 6.7 to 6.0, the label changed its color to green yellow and also the chitosan label changed its color from orange red to yellow. When pH decreased to 5.2 at 5th day of storage, cellulose film label changed from green yellow to yellow purple in color and chitosan containing label changed its color from yellow to purple red. On 7th day when milk was spoiled, cellulose containing label color turned to brown is shown in figure 1 and chitosan containing label also turned to brown is shown in Figure 2.

***Microbial and acidity analysis of milk*:** The maximum and minimum values of TPC observed during the studies were 5.73±0.02 and 4.84±0.01 respectively. A significant increasing trend in the total plate count was observed in the research. The milk containing labels displayed a minimum TA (%) on the 1st day of T_2 when it was fresh and as the storage progressed, a maximum TA (%) was noticed in T_2 on the 7th day. As storage progressed, an increase in acidity was noticed due to a decrease in pH of milk.

***Labels analysis*:** The mean value of present research data shows that the release of dye of T_1 was 0.20 and T_2 was 0.22 presented in table 2 which clearly declared that the mean value of cellulose pH indicator was less than chitosan indicator. The outcomes after statistical analysis indicated that the films' thickness, swelling index, tensile strength and elongation at break (%) of pH labels show highly significant effect with the treatments.

***Proximate analysis*:** A significant decreasing trend in crude fat was observed in the research. The maximum value of fat content was observed in T_1 3.81±0.01 at 1st day of storage while the minimum value of fat content 3.69±0.01 was observed in T_2 at 7th day. The fiber content in milk containing labels fluctuated between 0.73±0.01 to 0.39±0.02, as given in Table 3. A significant decreasing trend was perceived in the protein content of milk as the storage time increased.

***Physicochemical analysis*:** The gravity content in milk containing labels fluctuated between 1.030±0.001 to1.040±0.001, as given in Table 4. Specific gravity did not play any role in the color changing activity of labels. A significant increasing trend in the lactose content was observed in the research. The lactose content increased in milk due to the increase in lipolysis.

Table 1. Mean values for effect of treatment on total plate count and acidity of milk containing pH indicator label.

Storage	Mean	
	Total plate count	**Acidity of milk**
Day 1	T_1=4.86 ±0.01^{e}	T_1=0.14±0.01^{ef}
	T_2=4.84±0.01^{f}	T_2=0.13±0.01^{f}
Day 3	T_1=4.88±0.01^{d}	T_1=0.16±0.01^{de}
	T_2=4.86±0.01^{ef}	T_2=0.14±0.01^{ef}
Day 5	T_1=5.20±0.01^{c}	T_1=0.18±0.02^{c}
	T_2=5.17±0.01^{c}	T_2=0.16±0.01^{d}
Day 7	T_1=5.74±0.02^{a}	T_1=0.22±0.01^{b}
	T_2=5.70 ±0.02^{b}	T_2=0.24±0.02^{a}

T_1=Cellulose based film; T_2=Chitosan based film

Table 2. Mean values for effect of treatment on label release test, film thickness, swelling index %, tensile strength and elongation at break% of pH indicator label.

	Mean				
	Label release test	**Film thickness**	**Swelling index %**	**Tensile strength**	**Elongation at break %**
T_1	0.20 ±0.01^{b}	1.28 ±0.01^{b}	38.07 ±0.11^{a}	47.64 ±1.40^{b}	44.70 ±1.112^{b}
T_2	0.22 ±0.01^{a}	1.43 ±0.03^{a}	37.85 ±0.14^{a}	56.20 ±0.81^{a}	49.50 ±0.943^{a}

T_1=Cellulose based film; T_2=Chitosan based film

Table 3. Mean values for effect of treatment on crude fat, crude fiber and crude protein of milk containing pH indicator label.

Storage	**Mean**		
	Crude fat	**Crude fiber**	**Crude protein**
Day 1	T_1=3.82±0.01^{a}	T_1=0.73±0.01^{a}	T_1=3.28±0.03^{a}
	T_2=3.79±0.01^{b}	T_2=0.70±0.02^{a}	T_2=3.25±0.02^{a}
Day 3	T_1=3.79±0.01^{b}	T_1=0.65±0.02^{b}	T_1=3.22±0.01^{b}
	T_2=3.75±0.01^{c}	T_2=0.62±0.02^{c}	T_2=3.20±0.02^{b}
Day 5	T_1=3.75±0.01^{c}	T_1=0.59±0.02^{c}	T_1=3.20±0.02^{b}
	T_2=3.73±0.01^{d}	T_2=0.55±0.02^{d}	T_2=3.17±0.02^{c}
Day 7	T_1=3.72±0.01^{d}	T_1=0.41±0.02^{e}	T_1=3.17±0.02^{c}
	T_2=3.69±0.01^{e}	T_2=0.39±0.02^{e}	T_2=3.11±0.01^{d}

T_1=Cellulose based film; T_2=Chitosan based film

Table 4. Mean values for effect of treatment on specific gravity and lactose content of milk containing pH indicator label.

Storage	**Mean**	
	Specific gravity	**Lactose content**
Day 1	T_1=1.032±0.001^{f}	T_1=4.65±0.02^{e}
	T_2=1.030±0.001^{f}	T_2=4.61±0.01^{f}
Day 3	T_1=1.035±0.001^{de}	T_1=4.70±0.01^{cd}
	T_2=1.034±0.001^{e}	T_2=4.68±0.01^{d}
Day 5	T_1=1.038±0.001^{bc}	T_1=4.73±0.01^{b}
	T_2=1.036±0.001^{cd}	T_2=4.71±0.01^{bc}
Day 7	T_1=1.040±0.001^{a}	T_1=4.77±0.02^{a}
	T_2=1.039±0.001^{ab}	T_2=4.78±0.01^{a}

T_1=Cellulose based film; T_2=Chitosan based film

DISCUSSION

The fabricated indicator developed from cellulose film displayed a change in color from pink to brown at the seventh day of storage, which was correlated with the pH of the milk, indicating the ability of cellulose label to inform not only about the spoilage time of milk but also the medium fresh stage. And the indicator developed from chitosan film changed its color from orange red to brown on the seventh day of spoilage. This agrees with previous findings of other research workers (Pereira *et al.*, 2015) whom developed an indicator for detecting spoilage of milk by adding pigments of anthocyanin found from red cabbage to film of chitosan-PVA. They observed a change in color of film when storage times increased.

After the label release test, the results determined that cellulose label was more efficient compared to chitosan label because more color is released from chitosan label into the stored milk. The lycopene pigment was released more in chitosan film than in cellulose film mainly due to the polarity of water-soluble lycopene pigment. The release of dye from pH indicator labels were accordance to earlier research of (Eshaghi *et al.*, 2020) in which pH-monitoring film was developed by using natural dye from beetroot for detecting the milk spoilage.

The difference in thickness of both films is due to the different nature of both films used. The significantly lower swelling determined the presence of confirm strong intermolecular interactions between the components of extract and chitosan, that give a few sites of hydrophilic for molecules of water to network with chitosan and would also decrease the electrostatic repulsion between chain of polymer as related to film of cellulose. Cellulose film exhibited a decrease in both the tensile strength and elongation at break due to the micro phase separation and the stiffness of cellulose film. As a result of it, Chitosan film is more favorable for milk spoilage determination.

As the duration of milk storage in the refrigerator is extended, there is a corresponding decrease in its pH value, resulting in an increase in acidity and total plate count. In another research in which they developed films from Tara gum-cellulose Nano crystal-based combined with anthocyanin from grape skin. They also demonstrated a similar decreasing trend in pH at different storage times (Ma and Wang, 2016).

The milk samples were also analyzed for their proximate analyses such as crude fat, crude fiber and crude protein and physiochemical analysis which include specific gravity and lactose content of milk.

***Conclusion*:** The present research utilized natural dye as a freshness indicator to demonstrate the alterations in refrigerated milk storage. The utilization of polystyrene layers, white alum, and natural pigments in tomato as a natural reagent on a cellulose substrate appears to be a cost-effective approach. Due to the natural and edible properties of the reagent, it is considered appropriate for application in packaging of food. The label designed for pH sensing shown sensitivity towards pH variations in buffer solutions. Furthermore, it displayed consistent alterations in color throughout the storage of milk under refrigerated conditions. The fabricated indicator developed from cellulose film displayed a change in color from pink to brown at the seventh day of storage, which was correlated with the pH of the milk, indicating the ability of cellulose label to inform not only

about the spoilage time of milk but also the medium fresh stage. And the indicator developed from chitosan film changed its color from orange red to brown on the seventh day of spoilage. This distinct characteristic of the label is due to the compatibility between the polymeric base, specifically cellulose and chitosan, and the colorant used that is tomato lycopene.

***CRediT author statement*:** R. Afzal conducted the trials and completed the experiments; M. Arslan prepared the draft; A. Hassan reviewed and finalized the draft.

***Conflict of interest*:** The authors declare no conflict of interest.

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***Consent to participate*:** N/A

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***SDGs addressed*:** Good Health and Well-being, Industry, Innovation, and Infrastructure, Responsible Consumption and Production

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